ROSAMICIN, A MACROLIDE WITH *IN VITRO* ACTIVITY AGAINST *UREAPLASMA UREALYTICUM*

AURIOL C. HILL and GRAHAME SUTTON*

Medical Research Council Laboratory Animals Centre,
Woodmansterne Road, Carshalton, Surrey SM5 4EF, England
*War Memorial Hospital, The Park, Carshalton, Surrey SM5 3DB, England

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A new macrolide antibiotic, rosamicin, was shown to have much greater activity *in vitro* against ureaplasmas isolated from humans than erythromycin or the tetracyclines tested. A marked ureaplasmacidal effect was also shown.

Ureaplasma urealyticum has been implicated in both genital disease and infertility in man, but it has not always proved very responsive to available antibiotics and an effective antibiotic treatment is urgently required.

BRUNNER *et al.*[1] showed that 57% of patients with acute or chronic prostatitis were infected with *U. urealyticum,* compared with only 14% of healthy controls (ureaplasmas never exceeded 10^2 colony-forming units per ml in the controls), and they considered it to be the causative organism in 29% of acute and 23% of chronic cases.

GUPTA *et al.*[2] and FRIBERG *et al.*[3] found higher concentrations of ureaplasmas in the genital tracts of sterile couples than in those of fertile controls.

There is evidence that ureaplasmas can be the causative organisms of 'non-specific' urethritis. TAYLOR-ROBINSON *et al.*[4] experimentally induced non-specific urethritis in two male volunteers by inoculating a culture of *U. urealyticum* into their urethras.

EVANS *et al.*[5] showed that 10% of ureaplasma isolates were resistant to tetracycline and that all of them were sensitive to erythromycin. Repeated passage increased the resistance to minocycline of three out of four strains. As the concentration required to inhibit growth increased with exposure time, it appears that tetracycline(s) have a static rather than a ureaplasmacidal effect.

FORD *et al.*[6] have also reported a case of non-specific urethritis associated with a ureaplasma strain which was resistant to tetracycline but which, however, responded to erythromycin.

The findings of SPAEPEN *et al.*[7] were also consistent with those of EVANS *et al.*[5], in so far as they found that increasing concentrations of the four tetracyclines tested were required to inhibit the ureaplasmas examined. Ureaplasmas could, however, be recovered seven days after treatment from 42% of 50 patients. In contrast to EVANS *et al.*[5] they showed that only 11.4% of the ureaplasma strains examined were inhibited by 4.0 μg/ml of erythromycin.

A new micromonospora-produced macrolide antibiotic, rosamicin, has shown good results against various mycoplasmas. WAGMAN *et al.*[8], who described it, also tested it against *M. gallisepticum*, and WAITZ *et al.*[9] examined its activity against four more mycoplasma species. These included four strains of *M. pneumoniae,* five strains of *M. orale,* five strains of *M. salivarium* and one strain of *M. hominis. M. hominis* was inhibited by 0.4 μg/ml of rosamicin and all other species were inhibited by a concentration of less than 2.0 μg/ml.

It therefore seemed worthwhile testing rosamicin against *U. urealyticum in vitro* and comparing it with the antibiotics in common use against this organism.

Materials and Methods

Antibiotic

Rosamicin was obtained from Schering-Plough, New Jersey and stored at +4°C. On the day of the test some powder was removed and incubated at 60°C for three hours, and then dissolved in 95% ethyl alcohol at 2 mg/ml. This solution was diluted 1 in 2 with distilled water giving 1 mg/ml of rosamicin. Minocycline (Minocin, Lederle), doxycycline (Vibramycin, Pfizer), erythromycin (Erythrocin, Abbott Laboratories) and spiramycin (Rovamycin, May and Baker) were also made up on the day of the test—as 1 mg/ml solutions in distilled water.

Source of Organism

Ureaplasma strains were isolated from female patients presenting with inflammatory lesions of the lower genital tract. None of these patients had been on antibiotic therapy for three months prior to sampling. Swabs taken from the cervix were put into bottles containing 5 ml of urea liquid medium. The bottles were transported to the laboratory within two hours and either titrated immediately or frozen at −70°C. The inoculated medium was diluted tenfold to at least 10^{-4} in urea medium. When a pH change was seen the culture was frozen in aliquots of 1 ml at −70°C. Ureaplasmas were identified by urease production and characteristic morphology on agar.

Cultural Medium

Liquid medium was prepared with 70% Difco PPLO broth, 20% horse serum, 10% yeast extract, 0.002% phenol red, 0.1% urea and 1 in 2,000 thallous acetate, pH 6.9. Medium was also prepared as described but containing no thallous acetate.

Metabolic Inhibition Test (MIT)

The MIT was carried out as described by PURCELL *et al.*[10] Each well of a microtitre plate was inoculated with 0.05 ml of medium; 0.05 ml of rosamicin 1 mg/ml was added to the first well and then double diluted for two rows (23 wells) leaving the 24th well with medium alone as a control; 0.1 ml of medium was then added to each well. The frozen mycoplasma suspensions were thawed and each diluted four times giving dilutions of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. For each dilution 0.05 ml was each added to two rows (24 wells). This gave one microtitre plate with each isolate of mycoplasma seeded with four dilutions of the organism.

Plates were incubated at 35°C and examined at 1, 2, 5 and 7 days. The same technique was carried out using the other antibiotics. Rosamicin was tested against twenty-one ureaplasma isolates, spiramycin against five and the other antibiotics against eight isolates.

Mixed Infections

Both an ureaplasma isolate and *M. hominis* isolate were seeded onto the same plates. These organisms were tested against rosamicin at four dilutions to examine the possible synergistic effect of mixed species. Two cases were natural mixed infections and in the third an ureaplasma isolated from one patient was mixed with *M. hominis* isolated from a different patient.

Ureaplasmacidal Effect

Duplicate plates were set up containing the different antibiotics, except spiramycin, and seeded with ureaplasmas diluted 10^{-2} as described. The plates were incubated, one for 2 days and one for 5 days. The contents (0.2 ml) of five wells showing inhibition of growth prior to the well with a pH change (indicating growth of organism) were each then transferred to bottles containing 7 ml of medium. This diluted the antibiotic 1 in 35 (0.2 ml in 7 ml) to a dilution which had already been shown not to inhibit growth. The bottles were incubated at 35°C for two weeks to see whether there were viable organisms present.

Comparative Tests

Tests were also carried out: with medium containing no thallous acetate; with rosamicin made up as a 1 mg/ml solution without prior heating of the powder and with rosamicin powder dissolved in distilled water only.

Results

The inhibition of ureaplasmas by the antibiotics tested is shown in Tables 1~5. The growth of ureaplasmas was inhibited by very low concentrations of rosamicin, ranging from 0.477 to less than 0.030 μg per litre, compared to the other antibiotics tested (12~15,625 μg per litre).

There was very little difference in inhibition between the various concentrations of ureaplasma. The inhibitory concentration of antibiotics when the test was read at 2 or 7 days dropped heavily with minocycline and doxycycline, but not with erythromycin and spiramycin, which showed lower titres initially than the former antibiotics. In many cases the organism was inhibited by the lowest concentration of rosamicin tested which made it difficult to assess the original level of inhibition.

Table 1. Inhibition of ureaplasmas by rosamicin.

Isolate	Concentration of rosamicin (μg/litre) which inhibited growth			
	Day 2		Day 7	
	Organism dilution			
	10^1	10^4	10^1	10^4
A	<0.030	<0.030	0.060	0.120
B	<0.030	<0.030	<0.030	<0.030
C	<0.030	<0.030	<0.030	<0.030
D	0.060	<0.030	0.120	<0.030
E	0.060	0.060	0.120	0.060
F	<0.030	<0.030	<0.030	<0.030
G	<0.030	<0.030	<0.030	<0.030
H	0.060	0.060	0.060	0.060
I	0.060	<0.030	0.060	0.060
J	<0.030	<0.030	0.060	<0.030
K	0.120	0.060	0.238	0.477
L	<0.030	<0.030	0.477	0.060
M	<0.030	<0.030	0.238	0.120
N	<0.030	<0.030	<0.030	0.060
O	0.120	0.120	0.238	0.477
P	0.060	0.060	0.238	0.238
Q	<0.030	<0.030	<0.030	<0.030
R	<0.030	<0.030	0.060	0.060
S	<0.030	<0.030	0.120	0.060
T	0.060	0.060		
U	0.060	<0.030		

Table 2. Inhibition of ureaplasmas by minocycline.

Isolate	Concentration of minocycline (μg/litre) which inhibited growth			
	Day 2		Day 7	
	Organism dilution			
	10^1	10^4	10^1	10^4
A	0.199	0.050	51.09	51.09
B	0.050	<0.025	408.7	51.09
C	12.77	0.799	817.3	817.3
D	12.77	<0.025	817.3	204.3
E	<0.025	<0.025	51.09	51.09
F	0.050	N.G.		N.G.
G	0.050	0.050		
H	204.3	0.100	408.7	12.771

N.G. = No growth in control

Table 3. Inhibition of ureaplasmas by doxycycline.

Isolate	Concentration of doxycycline (μg/litre) which inhibited growth			
	Day 2		Day 7	
	Organism dilution			
	10^1	10^4	10^1	10^4
A	0.051	0.051	13.20	26.40
B	1.650	<0.026	422.4	13.20
C	211.2	13.20	16.89	16.89
D	1.650	0.825	16.89	211.2
E	0.412	<0.026	422.4	105.6
F	<0.026	N.G.		N.G.
G	<0.026	<0.026		
H	211.2	1.650	422.4	105.6

N.G. = No growth in control

Table 4. Inhibition of ureaplasmas by erythromycin.

Isolate	Concentration of erythromycin (μg/litre) which inhibited growth			
	Day 2		Day 7	
	Organism dilution			
	10^1	10^4	10^1	10^4
A	463.8	3.623	231.9	28.99
B	28.99	0.909	57.98	28.99
C	57.98	57.98	57.98	57.98
D	463.8	7.247	231.9	57.98
E	115.9	14.49	927.7	57.98
F	57.98	0.909	57.98	28.99
G	57.98	57.98	57.98	57.98
H	463.8	115.9	927.7	57.98

Table 5. Inhibition of ureaplasmas by spiramycin.

Isolate	Concentration of spiramycin (μg/litre) which inhibited growth			
	Day 2		Day 7	
	Organism dilution			
	10^1	10^4	10^1	10^4
A	976.6	488.3	3,906	7,812
B	3,906	3,906	7,812	15,625
C	3,906	7,812	15,625	15,625
D	15,625	3,906	31,250	7,812
E	31,250	1,953	31,250	7,812

Table 7. Ureaplasmacidal effect of antibiotics against ureaplasmas.

Isolate	Antibiotic	Antibiotic concentration (μg/litre)	
		Day 2	Day 5
A	Rosamicin	G	0.0005
B	Rosamicin	G	0.477
D	Rosamicin	G	0.238
E	Rosamicin	G	0.060
A	Minocycline	G	408.7
B	Minocycline	G	102.2
D	Minocycline	G	817.3
E	Minocycline	G	408.7
A	Doxycycline	G	γ
B	Doxycycline	G	844.7
D	Doxycycline	G	13,515
E	Doxycycline	G	β
A	Erythromycin	463.8	927.7
B	Erythromycin	231.9	463.8
D	Erythromycin	463.8	927.7
E	Erythromycin	463.8	463.8

G=Growth in all bottles.
γ=Growth in all bottles.
Lowest dilution taken 844.7 μg/litre
β=Growth in all bottles.
Lowest dilution taken 3,379 μ/litre

Table 6. Inhibition of ureaplasmas in a mixed infection of ureaplasma and *M. hominis* by rosamicin.

Isolate		Concentration of rosamicin (μg/litre) which inhibited growth			
		Day 2		Day 7	
Ureaplasma	*M. hominis*	Organism dilution			
		10^1	10^4	10^1	10^4
E	E	0.060	0.060	0.477	0.120
J	J	0.030	0.030	0.060	0.030
M	V	0.060	0.060	0.238	0.120

The mixed infection of ureaplasma and *M. hominis* made no significant difference to the inhibitory effect of rosamicin (Table 6). Similarly, no difference was shown in the titre with medium either containing or without thallous acetate.

The ureaplasmacidal effect of the antibiotics is shown in Table 7. Doxycycline needed a higher concentration of antibiotic than minocycline and was variable between isolates. Rosamicin showed a very good, though variable ureaplasmacidal effect with the four isolates tested, the highest concentration required was 0.477 μg per litre.

Discussion

Rosamicin gave a much better inhibitory and ureaplasmacidal effect *in vitro* than the other antibiotics tested. Erythromycin showed a moderate inhibition against ureaplasmas which did not significantly diminish with time and appeared to be ureaplasmacidal. These results are consistent with those of EVANS *et al.*[5] Minocycline and doxycycline initially showed very good activity which then rapidly dropped to lower levels than erythromycin, in agreement with EVANS *et al.*[5] The ureaplasmacidal effect was poor, particularly with doxycycline. The effect of spiramycin was not further tested as its inhibitory activity was lower than that of the other antibiotics.

Rosamicin was consistently very inhibitory to all 21 isolates tested. As only a few isolates were tested against the other antibiotics, strain variability could not be assessed. Concentration of the organisms did not generally make much difference to the result after a period of time.

Rosamicin was equally effective against ureaplasmas when they were mixed with *M. hominis*. The activity of rosamicin against *M. hominis* was greatly diminished compared to its activity against ureaplasmas and was consistent with the findings of WAITZ *et al.*[9]

Although rosamicin has shown very good action against ureaplasmas, it remains to be seen whether this drug is as effective *in vivo* as it is *in vitro*.

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